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The modulatory role of vascular endothelium in the interaction of agonists and antagonists with α-adrenoceptors in the rat aorta

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1 We have examined the effect of endothelium on the antagonistic action of prazosin, doxazosin, yohimbine and phentolamine against phenylephrine, clonidine and noradrenaline.

2 The action of prazosin against phenylephrine was similar to that earlier reported against noradrenaline, acting as a non-competitive antagonist in the presence of endothelium and as a competitive antagonist in the absence of endothelium. Prazosin also acted as a non-competitive antagonist against clonidine in the absence of endothelium.

3 Doxazosin behaved in a similar way to prazosin against noradrenaline, phenylephrine and clonidine acting as a non-competitive antagonist in the presence of endothelium and as competitive antagonist after removal of endothelium. In contrast, yohimbine and phentolamine acted as competitive antagonists both in the presence and in the absence of endothelium.

4 Analysis of the concentration-response curves for noradrenaline, phenylephrine and clonidine in the presence and in the absence of endothelium showed that the affinity for all three agonists was the same but not the efficacy and the receptor reserve, both of which were lower in the presence than in the absence of endothelium.

5 The rank order of agonist potency in the absence of endothelium was noradrenaline > phenylephrine > clonidine. The rank order of antagonist potency was prazosin ≥ doxazosin > phentolamine > yohimbine.

6 The results show that vascular endothelium modulates the contractile response to α-adrenoceptor agonists and also modifies the action of the antagonists prazosin and doxazosin but not that of yohimbine and phentolamine. This effect of endothelium was related to a change in agonist efficacy and receptor reserve. These results also suggest that the α-adrenoceptors of the isolated aorta of the rat are predominantly, if not exclusively of the α_1-subtype.

Introduction

Furchgott & Zawadzki (1980) first demonstrated the importance of vascular endothelium for the relaxant effect of acetylcholine in blood vessels via the release of a factor referred to as the endothelium derived relaxing factor (EDRF). Since then the role played by vascular endothelium in pharmacological and physiological responses of many blood vessels has been the subject of intense scientific investigation. Reports in this domain have shown that vascular endothelium not only mediates the relaxant responses to a wide range of vasodilator agents (reviewed by Furchgott, 1984), but also modulates the vasoconstrictor responses to many agonists (Allan *et al.*, 1983; Cocks & Angus, 1983; Eglème *et al.*, 1984; Godfraind *et al.*, 1985). It has also been proposed that the EDRF responsible for these effects is nitric oxide (NO) (Palmer *et al.*, 1987).

Recently we have reported that in rat isolated aorta, the modulation by endothelium of agonist-induced contractile response is related to an increase of cyclic GMP levels in vascular smooth muscles evoked by the release of EDRF, which in turn could result in a reduction of agonist efficacy and receptor reserve (Alosachie & Godfraind, 1986). This suggestion is in agreement with Malta *et al.* (1986) and Martin *et al.* (1986) who have also proposed a similar relation between endothelium modulation of agonist-induced contractile response and a possible

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alteration of agonist efficacy by EDRF. We have also shown that vascular endothelium modifies the mode of antagonism by prazosin of the noradrenaline concentration-response curve, where prazosin acted as non-competitive antagonist in the presence of endothelium and as a competitive antagonist in the absence of endothelium, a finding which could also be explained by a reduction in agonist efficacy and receptor reserve by EDRF (Alosachie & Godfraind, 1986).

The present study was designed to extend further our observations regarding endothelium modulation of prazosin antagonism by use of agonists of different α_1/α_2 selectivity with partial and full agonistic effect and by comparison of prazosin antagonism with that of doxazosin (selective α_1-antagonist), yohimbine (selective α_2-antagonist) and phentolamine (non-selective α-antagonist) under the same conditions. At the same time we aimed to investigate further the possible relationship between EDRF-induced changes in agonist efficacy and receptor reserve and the modulation by endothelium of agonist responses and prazosin antagonism. We have also attempted to characterize the post-synaptic α-adrenoceptors present in rat isolated aorta by considering the order of potency of agonists and of antagonists.

The results show that vascular endothelium modulates both α-adrenoceptor agonist responses and the mode of antagonism of these responses by prazosin and doxazosin but not by phentolamine and yohimbine. The results provide further evidence that this effect of EDRF is related to changes in efficacy and receptor reserve. On the other hand, the present observation also indicates that the characteristics of post-synaptic α-adrenoceptor population in the rat aorta, determined in the absence of the endothelial factor, are of the α_1-subtype.

Methods

Experimental protocol

Pairs of rings (2 mm long) were cut from the thoracic aorta of male Wistar rats (300–350 g). One ring of each pair was left intact, while the other was stripped of its endothelium by mechanical rubbing. Each ring was carefully suspended under a tension of 2 g in a 50 ml organ bath containing a physiological solution (mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 1.25, and glucose 11.5) at 37°C gassed with a mixture of 95% O_2 and 5% CO_2.

Contractile responses were measured with isometric strain gauges coupled to potentiometric pen recorders. After an equilibration period of 60 min the artery preparations were contracted maximally either by a depolarizing medium (composition mM: NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) or by noradrenaline (1 μM) when the agonist subsequently used was noradrenaline. Preparations were then washed and allowed a further 60 min period of equilibration. Absence of endothelial cells was demonstrated by the failure of the preparation to relax to acetylcholine (1 μM) which was applied when the initially evoked contraction was stable.

Cumulative concentration-response curves (CRCs) to various agonists were obtained by increasing the organ bath concentration of the agonist in steps of approximately 3 fold (Van Rossum, 1963), allowing equilibrium to be attained at each concentration. When the maximum effect was attained, preparations were washed and allowed a further 60 min period of equilibration. In experiments examining the effects of an antagonist, after the first CRC the tissue was incubated with the antagonist for 30 min. A second cumulative CRC was then constructed in the presence of the antagonist.

A pair of rings taken from the same aorta, one with and one without endothelium, were treated simultaneously as described above but without incubation with an antagonist; these served as controls to which all subsequent results were compared.

Analysis of results

The EC_{50} values were determined from individual CRCs by regression analysis (over the range 20–80% of the maximum response), the agonist was expressed by pD_2 values which were calculated as the negative logarithm of the EC_{50} value, the relative potency was calculated with reference to the EC_{50} of noradrenaline in the absence of endothelium.

For the assessment of the effect of prazosin, yohimbine and phentolamine at least three different concentrations of the antagonist were examined, one concentration of the antagonist was studied per aortic ring and the results were related to those obtained simultaneously in control rings from the same rat. pA_2 values for the antagonists were calculated from Arunlakshana & Schild (1959) plots constructed as log (dose-ratio − 1) against log (antagonist concentration). Dose-ratios were calculated at the EC_{50} level. Antagonism was considered to be competitive if there was not more than a 10% reduction in the maximum response and if the 95% confidence limits for the slope of the Schild plot, drawn by linear regression, overlapped unity. The affinity of the agonist for its receptors (K_A value) was calculated according to the method of Furchgott & Bursztyn (1967) (see also Alosachie & Godfraind, 1986).

The K_A value thus determined was used in the following equation (1) to calculate the fraction (R_A/R_t)

Table 1 Comparison of the characteristics of concentration-response curves of α-adrenoceptor agonists in rat isolated aorta in the presence and absence of endothelium

Compound	*Endo.*	n[a]	EC_{50}	pD_2	E_{max} (mg)[b]	*Relative potency*[c]
Noradrenaline	present	36	$3.1 \pm 0.5 \times 10^{-8}$	7.51	2822 ± 128	0.21
	absent	40	$6.5 \pm 0.3 \times 10^{-9}$	8.39	3034 ± 212	1.00
Phenylephrine	present	34	$4.2 \pm 0.6 \times 10^{-8}$	7.38	2639 ± 158	0.15
	absent	38	$1.1 \pm 0.3 \times 10^{-8}$	7.96	3094 ± 154	0.59
Clonidine	present	8	$1.1 \pm 0.1 \times 10^{-7}$	6.96	284 ± 34	0.06
	absent	36	$3.3 \pm 0.2 \times 10^{-8}$	7.48	2609 ± 130	0.20

[a] Number of cumulative concentration-response curves used in the calculation.
[b] Maximal contractile response expressed in mg of isometric tension.
[c] The relative potency calculated with reference to the EC_{50} of noradrenaline (endothelium absent).

of receptors occupied at each concentration of the agonist in the intact and denuded preparations

$$R_A/R_t = \frac{[A]}{K_A + [A]} \quad (1)$$

where (R_A) is the concentration of receptor-agonist complex and (R_t) is the total receptor concentration. The control concentration-response curves for the agonist in the presence and absence of endothelium were then plotted as a function of the negative logarithm of the fractional receptor occupation (R_A/R_t) and the appropriate curves were constructed.

The relative efficacy of each agonist in the presence and absence of endothelium was calculated as the antilog of the distance along the abscissa between the receptor-occupancy response curves for noradrenaline in the absence of endothelium and the agonist curve. For more details see Furchgott & Brusztyn (1967).

Drugs

Noradrenaline bitartrate (Flücka) was dissolved in distilled water containing 7.9 mM Na_2SO_3 and 34 mM HCl as a stock solution of 10 mM. Prazosin (Pfizer), doxazosin (gift from Pfizer) and phenoxybenzamine (gift from Janssen Pharmaceutica) were dissolved in ethanol and diluted in distilled water to 10 mM (final ethanol concentration 15%). Phenylephrine HCl (Sigma), clonidine HCl (Boehringer), yohimbine HCl (Sigma) and phentolamine mesylate (Ciba) were dissolved in distilled water.

Statistical analysis

The data are expressed as mean ± s.e.mean. Tests for significance have been made using Student's *t* test, *P* values less than 0.05 being considered significant. A least squares linear regression analysis was used to fit straight lines to data when appropriate. Experimental data were analysed for statistical differences by variance analysis wherever necessary *P* values less than 0.05 being considered significant.

Results

Comparison of concentration-response curves to α-adrenoceptor agonists in the presence and absence of endothelium

A comparison was made of the effect of the following α-adrenoceptor agonists on intact and denuded preparations of rat isolated aorta: noradrenaline, a non selective α-adrenoceptor agonist; phenylephrine, a preferential α_1-adrenoceptor agonist; clonidine, a preferential α_2-adrenoceptor agonist.

The characteristics of the CRCs to noradrenaline, phenylephrine and clonidine were compared in the presence and absence of endothelium (Table 1); removal of endothelium significantly decreased the EC_{50} values, increased maximal responses and relative potencies for all three agonists especially for clonidine which had only a weak contractile response in the presence of endothelium, confirming our earlier observations (Godfraind *et al.*, 1985).

Comparison of the antagonism of contractile responses to noradrenaline, phenylephrine and clonidine by prazosin, doxazosin, yohimbine and phentolamine in the presence and absence of endothelium

In preparations with intact endothelium, the action of prazosin against phenylephrine was similar to that earlier reported, against noradrenaline: prazosin produced a concentration-dependent non-parallel shift to the right of the CRCs to phenylephrine with a progressive depression of the maximal response (Figures 1 and 2) indicating a non-competitive

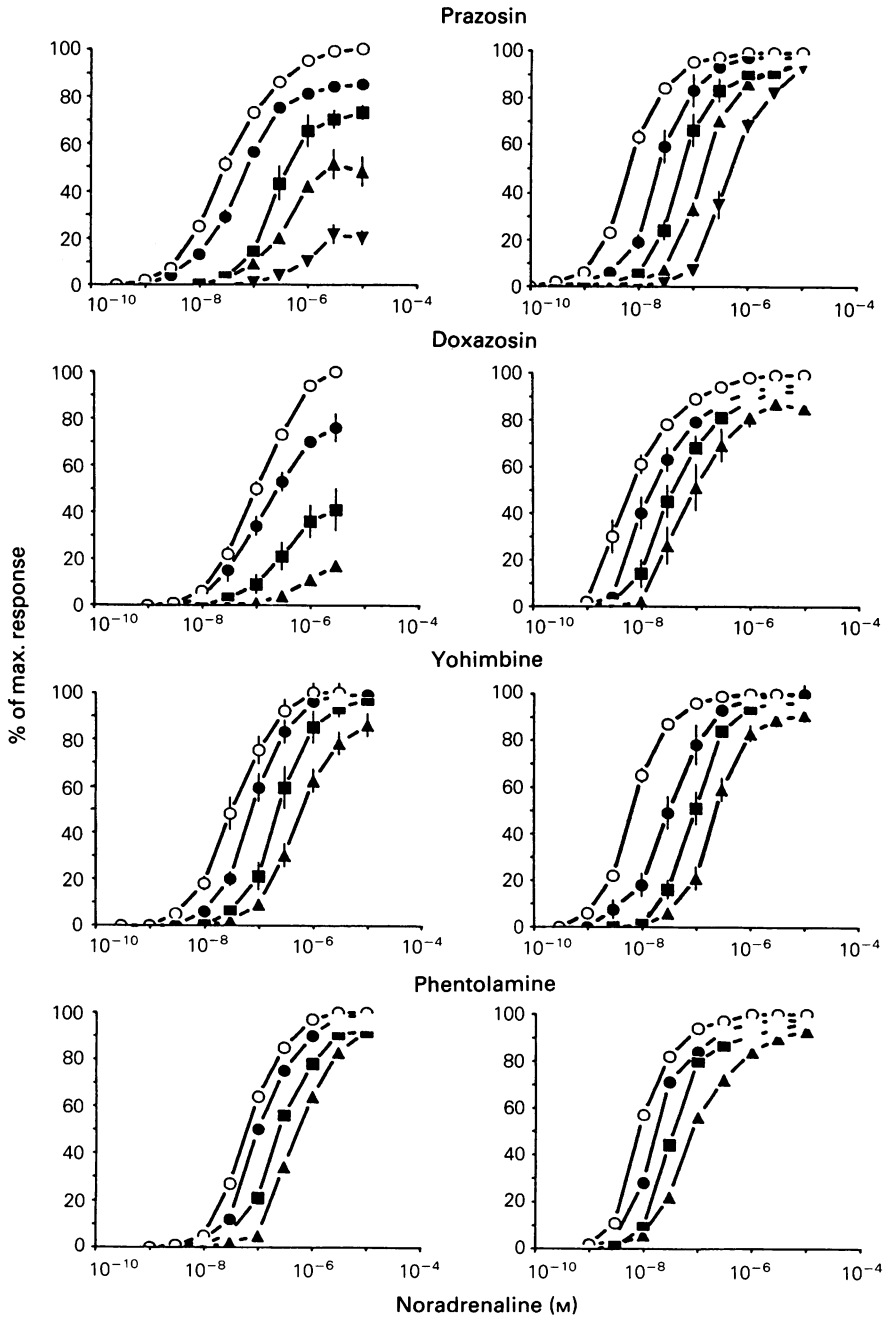

Figure 1 Antagonism of the concentration-response curves to noradrenaline by prazosin (● 0.3 nM; ■ 1 nM; ▲ 3 nM; ▼ 10 nM), doxozasin (● 0.3 nM; ■ 1 nM; ▲ 3 nM), yohimbine (● 0.3 μM; ■ 1 μM; ▲ 3 μM) and phentolamine (● 1 nM; ■ 3 nM; ▲ 10 nM) in the presence (left panel) and in the absence (right panel) of endothelium. Open circles (○) represent control responses to noradrenaline. Each point represents the mean and vertical lines show s.e.mean ($n > 4$).

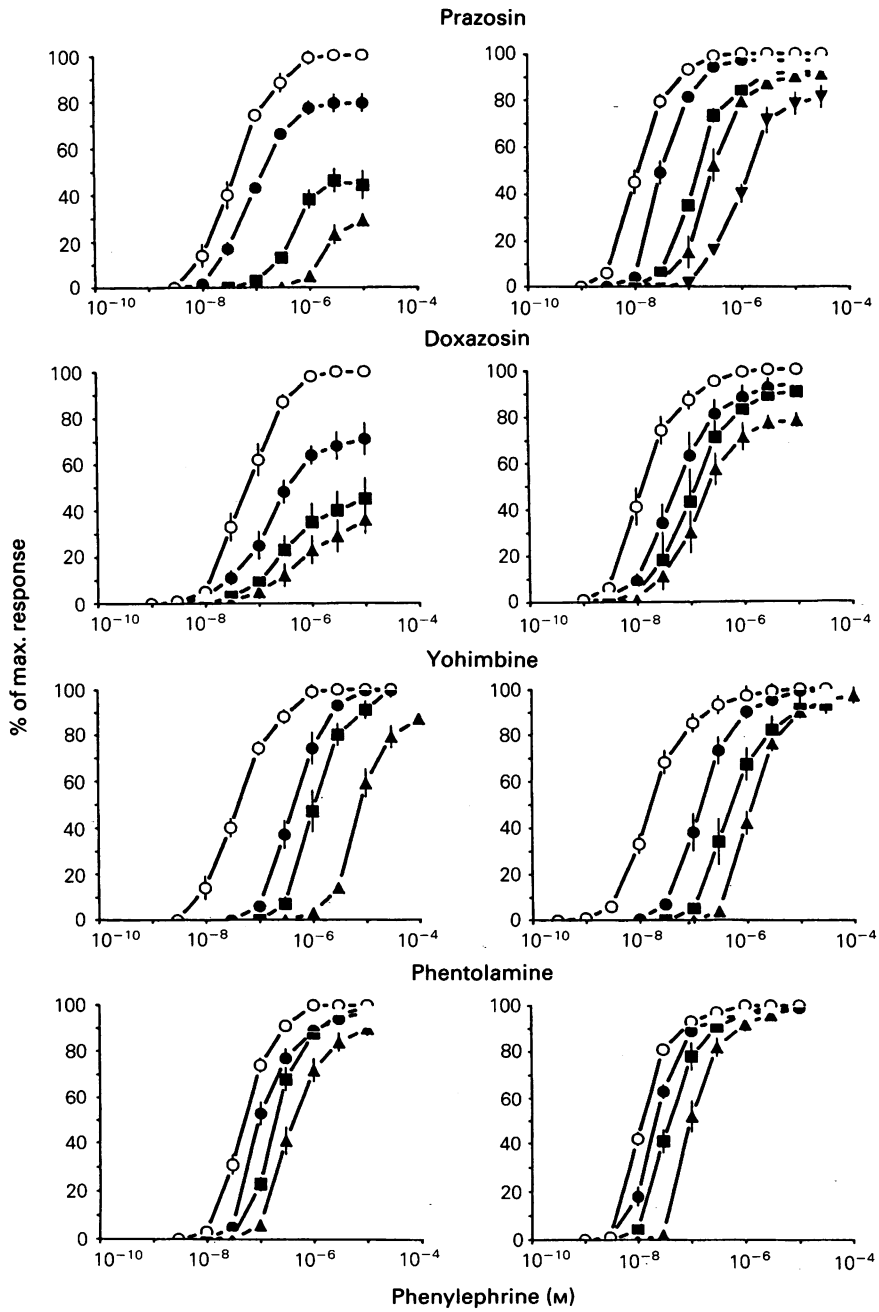

Figure 2 Antagonism of the concentration-response curves to phenylephrine by prazosin (● 0.3 nM; ■ 1 nM; ▲ 3 nM; ▼ 10 nM), doxazosin (● 0.3 nM; ■ 1 nM; ▲ 3 nM), yohimbine (● 1 μM; ■ 3 μM; ▲ 10 μM) and phentolamine (● 1 nM; ■ 3 nM; ▲ 10 nM) in the presence (left panel) and in the absence (right panel) of endothelium. Open circles (○) represent control responses to phenylephrine. Each point represents the mean and vertical lines show s.e.mean ($n > 4$).

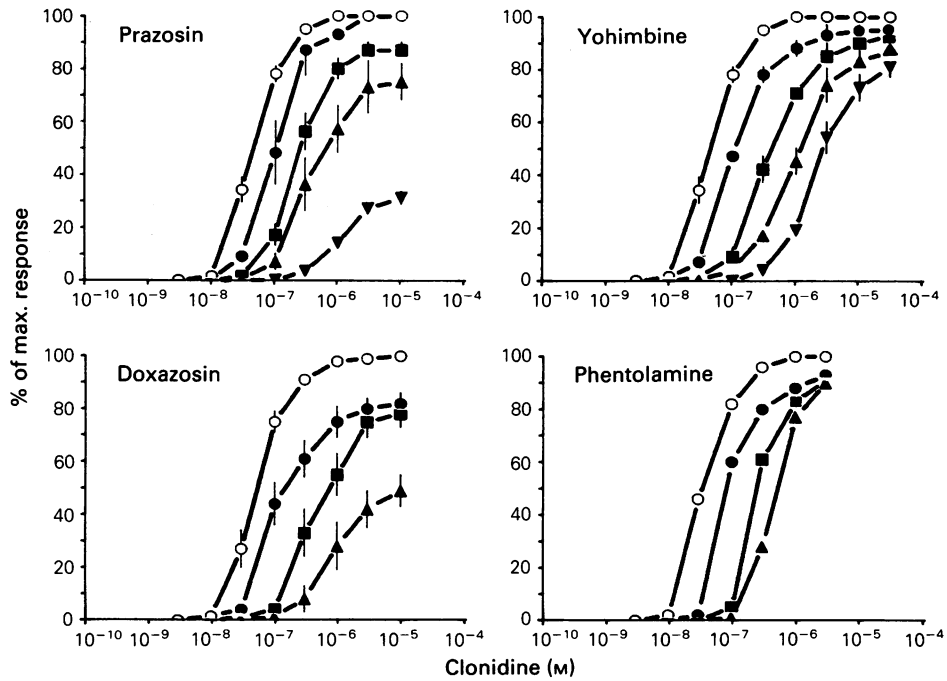

Figure 3 Antagonism of the concentration-response curves to clonidine by prazosin (● 0.1 nM; ■ 0.3 nM; ▲ 1 nM; ▼ 3 nM), doxazosin (● 0.3 nM; ■ 1 nM; ▲ 3 nM), yohimbine (● 0.1 μM; ■ 0.3 μM; ▲ 1 μM; ▼ 3 μM) and phentolamine (● 1 nM; ■ 3 nM; ▲ 10 nM) in the absence of endothelium. Open circles (○) represent control response to clonidine. Each point represents the mean and vertical lines show s.e.mean ($n > 4$).

antagonism. Doxazosin acted similarly to prazosin, producing a concentration-dependent non-parallel shift to the right of the control CRCs to both noradrenaline and phenylephrine with a progressive depression of the maximal response (Figures 1 and 2). However, yohimbine and phentolamine produced a concentration-dependent parallel shift to the right of CRCs to noradrenaline and phenylephrine with no significant depression of the maximal response. The slope of the Schild plot was non-significantly different from unity indicating a competitive antagonism (Figures 2 and 3). The contractile activity of clonidine in the presence of endothelium was too low to perform such an experiment.

In preparations where endothelium was removed, at concentrations between 0.3 to 10 nM, prazosin and doxazosin treatment resulted in a concentration-dependent, parallel shift to the right of the CRCs to noradrenaline and phenylephrine with no significant depression of the maximal response (concentrations higher than 10 nM evoked a significant depression of the maximum, data not shown). The slopes of the Schild plot were not significantly different from unity, indicating a competitive antagonism (Figures 1 and 2). Prazosin and doxazosin at the same dose-range evoked a concentration-dependent non parallel shift to the right of the CRCs to clonidine with a progressive depression of the maximal response, indicating a non competitive antagonism (Figure 3), whereas yohimbine and phentolamine evoked a concentration-dependent, parallel shift to the right of the CRCs to all three agonists with no significant depression of their maximal responses. The slopes of the Schild plot were not significantly different from unity, indicating a competitive antagonism.

Schild plots were constructed for each agonist against prazosin, doxazosin, yohimbine and phentolamine. pA_2 values together with characteristics of Schild plots were compared in the presence and absence of endothelium (Table 2).

Comparison of the agonist dissociation constants for noradrenaline, phenylephrine and clonidine in the presence and absence of endothelium

The agonist dissociation constant (K_A) for noradrenaline and phenylephrine, in the presence and absence of endothelium and for clonidine in the absence of endothelium were determined according

Table 2 Comparison of mean pA_2 values and characteristics of Schild-plots of prazosin, doxazosin, yohimbine and phentolamine against α-adrenoceptor agonists between intact and rubbed preparations of isolated aorta of the rat

		Endothelium present			*Endothelium removed*		
Agonist	*Antagonist*	*pA_2*	*Slope*	r	*pA_2*	*Slope*	r
Noradrenaline	Prazosin				**9.97 ± 0.44	0.95 ± 0.07	0.99
	Doxazosin				9.63 ± 0.83	1.1 ± 0.15	0.99
	Yohimbine	6.66 ± 0.19	1.04 ± 0.12	0.99	7.08 ± 0.28	0.95 ± 0.9	1.00
	Phentolamine	8.32 ± 0.02	1.04 ± 0.05	0.99	8.51 ± 0.04	0.92 ± 0.07	0.99
Phenylephrine	Prazosin				9.79 ± 0.14	1.10 ± 0.13	0.99
	Doxazosin				10.02 ± 0.17	0.88 ± 0.14	0.99
	Yohimbine	6.93 ± 0.18	1.03 ± 0.13	0.99	6.96 ± 0.25	0.94 ± 0.16	0.99
	Phentolamine	8.41 ± 0.07	0.90 ± 0.11	0.99	8.39 ± 0.08	0.97 ± 0.02	1.00
Clonidine	Prazosin				*10.10 ± 0.14	0.81 ± 0.11	0.98
	Doxazosin				*9.66 ± 0.02	1.48 ± 0.03	1.00
	Yohimbine				7.17 ± 0.12	1.00 ± 0.11	0.99
	Phentolamine				8.65 ± 0.16	1.01 ± 0.15	0.99

* Apparent pA_2 values (not real) due to the non competitive nature of the antagonism calculated for comparative interest.

** pA_2 calculated from data taken from Alosachie & Godfraind (1986).

to the technique of Furchgott & Bursztyn (1967) (see Methods). For clonidine in the presence of endothelium, the above mentioned method was not applicable due to the very low contractile activity displayed by clonidine in such preparations. However an independent method was used to estimate the dissociation constant for clonidine in the presence of endothelium. Since the contractile agonist response to clonidine in intact preparations was so weak, almost negligible, clonidine was used as a competitive antagonist against noradrenaline and an estimate of the equilibrium constant for clonidine was obtained from a Schild plot (Furchgott & Bursztyn, 1967; Kenakin, 1984).

The dissociation constants for noradrenaline, phenylephrine and clonidine in the presence and absence of endothelium, in addition to their relative affinities calculated with reference to the affinity of noradrenaline to its receptor in the absence of endothelium are listed in Table 3. The agonist dissociation constants shown in Table 3 demonstrate that the removal of endothelium did not result in a significant change in their values. The dissociation constants for clonidine determined either by the method of Furchgott & Bursztyn or by using clonidine as a competitive antagonist to noradrenaline showed no significant difference.

Effect of endothelium on the relative efficacies and spare receptors for noradrenaline, phenylephrine and clonidine

Receptor-occupancy response curves for noradrenaline, phenylephrine and clonidine were compared, in the presence and absence of endothelium. A marked shift to the left of the curves representing the contractile response of the rat aorta as a function of receptor occupation after endothelium removal is evident for all three agonists, indicating the presence of a higher receptor reserve in preparations without endothelium, since the leftward shift represents the decreasing amount of receptors necessary for a maximal response.

The relative efficacies for the agonists were determined in the presence and absence of endothelium. The values for the relative efficacy (e_R) are listed in Table 3 where we noted an increase in the efficacy of the agonists after removal of endothelium for all three agonists.

The agonist with the highest efficacy was noradrenaline in the absence of endothelium. The rank order of relative efficacy in the presence and absence of endothelium was the same: noradrenaline > phenylephrine > clonidine.

Discussion

Our previous observations on the effect of endothelium on prazosin antagonism against noradrenaline (Alosachie & Godfraind, 1986), have been extended to other agonists namely phenylephrine and clonidine. Prazosin behaved similarly against phenylephrine and noradrenaline acting as a non competitive antagonist in the presence of endothelium and as a competitive antagonist after removal of endothelium. A similar pattern was observed with doxazosin. For clonidine, such a comparison was not possible since

Table 3 Comparison of dissociation constants (K_A) relative affinities, relative efficacies for α-adrenceptor agonists in rat isolated aorta in the presence and absence of endothelium

Compound	*Endo.*	n[a]	K_A (M)	$-log\ K_A$	*Relative affinity*[b]	*Relative efficiency*[c]
Noradrenaline	present	9	$1.53 \pm 0.32 \times 10^{-7}$	6.82	0.95	0.19
	absent	11	$1.45 \pm 0.51 \times 10^{-7}$	6.84	1.00	1.00
Phenylephrine	present	10	$8.06 \pm 1.13 \times 10^{-8}$	7.09	1.78	0.10
	absent	10	$7.11 \pm 0.85 \times 10^{-8}$	7.15	2.04	0.35
Clonidine	present	9	$8.63 \pm 1.34 \times 10^{-8}$[d]	7.06	1.68	0.01
	absent	5	$6.36 \pm 0.96 \times 10^{-8}$	7.20	2.27	0.09

[a] Number of experiments.
[b] Relative affinity calculated with reference to K_A noradrenaline (endothelium absent).
[c] Relative efficacy with respect to noradrenaline (endothelium absent) calculated at a relative response of 0.5 of the respective agonist.
[d] Dissociation constant obtained for clonidine acting as a competitive antagonist against noradrenaline.

the contractile response to this agent in the presence of endothelium was very weak. In the absence of endothelium, prazosin and doxazosin acted as non competitive antagonists to clonidine which even in the absence of endothelium acts as partial agonist with a very small or no receptor reserve. This finding strengthened our view that the change in the mode of antagonism of prazosin after removal of endothelium was related to a difference in the receptor reserve between intact and rubbed preparations.

On the other hand, yohimbine and phentolamine acted as competitive antagonists against noradrenaline and phenylephrine in the presence and in the absence of endothelium and against clonidine in the absence of endothelium. Thus, endothelium did not affect the mode of antagonism of these two antagonists. The results of these experiments gave further support to our previous suggestion that prazosin could not be considered as a competitive antagonist since it acts as a competitive antagonist only within a certain dose-range not exceeding 10^{-8} M in the case of rat isolated aorta, providing there is a high receptor reserve. Once its concentration is increased or the receptor reserve decreases, it acts as a non competitive antagonist unlike phentolamine and yohimbine which were not sensitive to the decrease in receptor reserve in intact preparations.

On the basis of the higher sensitivity of clonidine and α_2-adrenoceptor agonists in general to the effect of endothelium, it has been suggested that these agonists may provoke the release of EDRF by an interaction with α_2-adrenoceptors located on the endothelium cells (Cocks & Angus, 1983; Eglème *et al.*, 1984; Miller *et al.*, 1984; Bullock *et al.*, 1986). However, in rat aorta the α-adrenoceptor agonist guanfacin does behave identically to clonidine (Godfraind *et al.*, 1985) and the α_1-adrenoceptor agonist St 587 does not behave similarly to noradrenaline or phenylephrine. In addition, rauwolscine an α_2-selective antagonist did not mimic the effect of the removal of endothelium (Lues & Schuman, 1984). Also attempts to induce endothelium-dependent relaxation in rat aorta, using clonidine after blocking α_1-adrenoceptors by prazosin have failed (Godfraind *et al.*, 1985; Martin *et al.*, 1986). Furthermore, no α_2 binding sites could be detected on the rat aortic smooth muscle or its endothelium by use of [^{3}H]-rauwolscine binding combined with autoradiography (Jacobs & Dashwood, 1986).

If we look at the α-adrenoceptor agonists whose maximal contractile responses are considerably reduced by endothelium, i.e. clonidine, oxymetazoline BHT 920 and St 587, we see that the common feature of these agents is that they all act as partial agonists in the isolated aorta of the rat (Ruffolo *et al.*, 1979; Diggs & Summers, 1983).

An alternative explanation to the interaction with α_2-adrenoceptors on the vascular endothelium could be proposed based on the concepts of efficacy and receptor reserve.

Curves representing the contractile responses to the agonists as a function of receptor occupancy (Figure 4) demonstrate some interesting features: (1) For all three agonists the receptor occupancy-response curves were shifted to the left after removal of endothelium indicating a higher receptor reserve in the absence of endothelium. (2) In the absence of endothelium, receptor occupancy-response curve for clonidine shows a linear relationship indicating very low or no receptor reserve which is a feature consistent with the partial agonist nature of clonidine. (3) In the presence of endothelium the receptor occupancy response curve for phenylephrine overlaps that for clonidine after endothelium removal, also manifesting a linear relationship between receptor

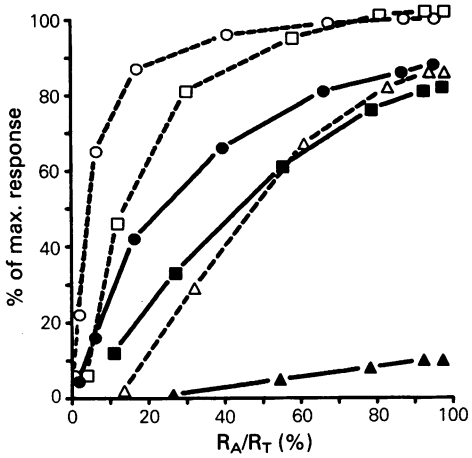

Figure 4 Contractile response to α-adrenoceptor agonists as a function of receptor occupation compared in the presence and absence of endothelium. Curves are for noradrenaline in the presence (●) and in absence (○) of endothelium; phenylephrine in the presence (■) and in the absence (□) of endothelium; clonidine in the presence (▲) and in the absence (△) of endothelium. Contractile responses are expressed as a percentage of the maximal response to noradrenaline in the absence of endothelium.

occupancy and contractile response which indicates very low or no receptor reserve for phenylephrine in the presence of endothelium. Receptor occupancy-response curves for noradrenaline in the presence of endothelium manifested an hyperbolic relation between receptor occupation and contractile response, indicating the presence of a considerable amount of receptor reserve, although much lower than that estimated in the absence of endothelium.

The relative efficacies of the agonists listed in Table 3, show that in the presence of endothelium, the relative efficacy for all three agonists was lower than in the absence of endothelium. On the basis of these estimates, in the absence of endothelium, noradrenaline would have to occupy only 1% of the receptors occupied by clonidine to induce the same contractile response elicited by clonidine in the presence of endothelium and if we compare the two agonists in the absence of endothelium, noradrenaline would still need to occupy less than 10% of the receptors occupied by clonidine to produce the same response (Figure 4).

The present results indicate that EDRF can be considered as an endogenous functional antagonist reducing the apparent efficacy of the adrenoceptor agonist. Partial agonists with low efficacy and low or no receptor reserve appear to be more sensitive to this effect of EDRF than full agonists with high efficacy for which a high receptor reserve might buffer the functional antagonistic effect of EDRF. This is in agreement with the observation that prazosin and doxazosin produced a greater depression of the contractile responses to noradrenaline and phenylephrine in the presence of endothelium. Indeed, receptor theory predicts that non-competitive antagonists produce greater inhibition in systems lacking receptor reserve (Ariens & van Rossum, 1957).

Another objective for carrying out the present experiments was to characterize the postsynaptic α-adrenoceptors present in the isolated aorta of the rat, the exact identity of which has been controversial (e.g. Timmermans & van Zwieten, 1981; Ruffolo *et al.*, 1981; Randriantsoa *et al.*, 1981). Most of the data available at present were obtained from studies that did not take into account the endothelial factor.

Comparison of the potency and intrinsic activity of noradrenaline, phenylephrine and clonidine, in rat isolated aorta in the absence of endothelium, shows that the rank order of relative potency was noradrenaline > phenylephrine > clonidine (Table 1). This order of potency is indicative of an α_1-type of adrenoceptor (Starke & Docherty, 1980).

The rank order of potency of the antagonists, interpreted from pA_2 values (Table 2) is prazosin > phentolamine > yohimbine. All three of these antagonists had similar pA_2 values against noradrenaline, phenylephrine or clonidine suggesting that only a single population of adrenoceptors was present. The pA_2 values for prazosin (approximately 10) compared to pA_2 values for yohimbine (approximately 7), show an approximately 1000 fold difference in affinity between prazosin (selective α_1) and yohimbine (selective α_2) which with the rank order of potency of antagonists indicates an α_1-adrenoceptor subtype.

The dissociation constant for agonist provides an estimate of the affinity of the agonist for its receptor, irrespective of the presence of a receptor reserve. Although the values of the affinity constants are somewhat different from those of Ruffolo *et al.* (1979) and Ruffolo & Waddell (1982), especially for phenylephrine which was significantly lower in our hands, a reasonable agreement exists with respect to rank order of affinity for the three agonists which in this study, was: clonidine > phenylephrine > noradrenaline.

Though clonidine is a preferential α_2-adrenoceptor agonist *in vitro* it exerts considerable α_1-adrenoceptor activity as the present study demonstrates, confirming studies by Diggs & Summers (1983) and Hamed *et al.* (1983).

The relative efficacy calculated for each agonist with respect to noradrenaline (Table 3) shows that the rank order of efficacy was noradrenaline > phenylephrine > clonidine. Plotting the agonist

responses against receptor occupation indicates the efficiency of the agonist receptor interaction (Figure 4). In this case, clonidine, the weaker partial agonist, shows a linear relation between its response and receptor occupation while the full agonists, noradrenaline and phenylephrine, show a hyperbolic relation.

The evaluation of the above parameters in the rubbed preparations of rat isolated aorta strongly suggests that the properties of the adrenoceptors present are mostly of the α_1-adrenoceptor subtype, which is in agreement with previous studies (Mcgrath, 1982; Diggs & Summers, 1983; Hamed *et al.*, 1983).

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